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The expression level of miRNA-601 in the serum of Iranian lung cancer patients and its association with smoking

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Abstract

Introduction: Lung cancer is a devastating disease that is often diagnosed at an advanced stage, leading to poor prognosis and limited treatment options. Therefore, the identification of reliable biomarkers for early detection and monitoring of lung cancer is critical. In this study, we investigated the expression level of miRNA-601 in the serum of Iranian lung cancer patients and its relation to smoking.

Methods: This study analyzed serum samples of 40 patients with lung cancer, and serum samples of 40 healthy individuals. Patient samples consisted of individuals who were at all stages, stages one, two, three, and four. In this study, miRNA was extracted from serum with Trizol solution. Then, using a cDNA synthesis kit, cDNA was constructed, and using the Rael Time PCR method, the expression of this miRNA was investigated.

Results: There was no significant difference in the expression level of miRNA-601 between control and patients with tumor stage I, II and III, but miRNA-601 expression was significantly downregulated in patients with tumor stages IV ($P<0.05$). A significant, negative relationship was found between miRNA-601 expression and tumor stage ($P<0.001$). We observed that 72% of patients with stages III and IV NSCLC had a positive smoking history.

Conclusion: our study underscores the importance of identifying reliable biomarkers for the detection and monitoring of lung cancer.

Keywords: lung cancer, microRNA, smoking

1. Introduction

Lung cancer is one of the leading causes of cancer related death worldwide and its 5-year survival rate is low, about 15%-20%[1]. Although air pollution and radon exposure also play a part in the development of LC, tobacco use continues to be the predominant risk factor[2]. An imaging test, such as a chest X-ray, CT scan, or MRI, together with a bronchoscopy, biopsy, or other tissue sample analysis, is frequently used to diagnose lung cancer [3].

LC can be divided into two types: small cell lung cancer (SCLC) with a neuroendocrine origin and non-small cell lung cancer (NSCLC) [4]. Approximately 80% of lung cancer cases are NSCLC, a heterogeneous class of tumors [5, 6]. NSCLC is divided into three main types: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy are all possible treatments for NSCLC[7].

A class of non-coding RNAs known as microRNAs has the ability to control gene expression by directly cutting mRNA or by inhibiting translation by binding with the target mRNA. Many of these molecular structures can function as an oncogene or tumor suppressor, and they have been engaged in managing the physiological and pathological processes of the cell [8, 9]. A lot of research has been done on the microRNAs and their role in cancer and found that microRNAs can be good biomarkers for the treatment and

diagnosis of cancer, the expression of the inaccurate microRNAs can cause cancer [10]. The microRNA601 plays a role in cellular, apoptotic and cellular function, and the microbial end of this microRNA disrupts cellular configurations [11]. In this study, due to the prognosis of the diagnosis of metastasis in lung cancer, the present study was designed to use microRNA601 as a biomarker for the diagnosis of lung cancer.

2. Materials and methods

2.1. Study population

Between April 2010 and September 2012, a total of forty patients who were newly diagnosed with non-small cell lung cancer (NSCLC) at the Masih Daneshvari Hospital in Tehran, Iran was recruited for the study. The mean age of the patients was 57.9 ± 9.5 years, where SD represents the standard deviation. Histopathological and clinical evaluations corroborated the manifestation of pulmonary carcinoma, while the subjects remained untreated and had no past medical history of neoplastic or inflammatory pathologies. These individuals underwent a general health evaluation and reported no history of cancer or inflammatory ailments. In the present investigation, a sample of 40 patients was subjected to random assignment, with 27 male individuals and 13 females included in the final cohort.

2.2 Serum sample collection

A volume of 5 milliliters of blood samples was acquired from fasting individuals and transferred into sterile tubes that were supplemented with the anticoagulant, ethylenediaminetetraacetic acid (BD Vacationer; Becton Dickinson and Company, Franklin Lakes, NJ) EDTA. the blood serum was separated at 4°C by centrifugation at 3,500 g for 10 min and stored at minus 80.

2.3 RNA extraction

Total RNAs from serum were extracted and purified using TRIzol (Invitrogen) following the manufacturer's instructions. In summary, TRIzol was added to serum which was then subjected to treatment with chloroform (from Merck, Germany) and followed by sedimentation with isopropanol (from Merck, Germany) and ethanol washing. The resultant total RNA was later diluted in water that had been treated with sterile DEPC.

2.4 Qualification and quantification of RNA

The RNA was evaluated by measuring and calculating its quality and quantity using a NanoDrop machine (manufactured by Thermo Fisher Scientific in the USA) and electrophoresis was conducted on a 1% agarose gel.

2.5 Reverse transcription

The RNA that was obtained was subjected to reverse transcription utilizing the miRCURY LNA Universal RT microRNA cDNA Synthesis Kit (miRCURY LNA RT Kit-QIAGEN, MD, USA) following the guidelines provided by the manufacturer.

2.6 Quantitative real-time polymerase chain reaction (qRT-PCR)

The present study utilized qRT-PCR to characterize miRNA-601 as the targets, and u6 as the reference gene. Table 1 lists the primers used for these genes. A total volume of the 20 µl qPCR reaction, which included 7 µl distilled water, 10 µl master mix, 2µl cDNA, and 1 µl primers was used. The amplification program was designed according to the appropriate annealing temperature: 1 cycle at 95°C for 60 seconds, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and 72°C for 30 seconds. Expression of the target miRNA-601 was normalized by miRNA REST software. all tests were performed in triplicate.

2.7 statistical analysis

Statistical analysis was performed using SPSS 19 software (SPSS Inc., Chicago, IL, USA) and the results were analyzed by one-way ANOVA. The expression level of target genes between the treated samples and control group was measured by Tukey's HSD post hoctest. Data were presented as mean ± standard deviation (SD) and $p < .05$ was considered statistically significant.

3.Results

3.1 RNA quantity and quality

The assessment of the purity, concentration, and integrity of total RNA was carried out through the use of 1% agarose gel electrophoresis. In our RNA gel electrophoresis analysis, we observed the presence of two distinct bands, which were identified to correspond to individual subunits of ribosomal RNA.

Table1. Primer sequences

MiR601	**Forward:** GAAAGACAAGCAGTGGACTG **Reverse:** GTGGGCTGGTTCCTTAAAGA
U6	**Forward:** GCTCGCTTCGGCAGCACATATAC **Reverse:** GGTCCGAGGTATTCGCACTGGATA

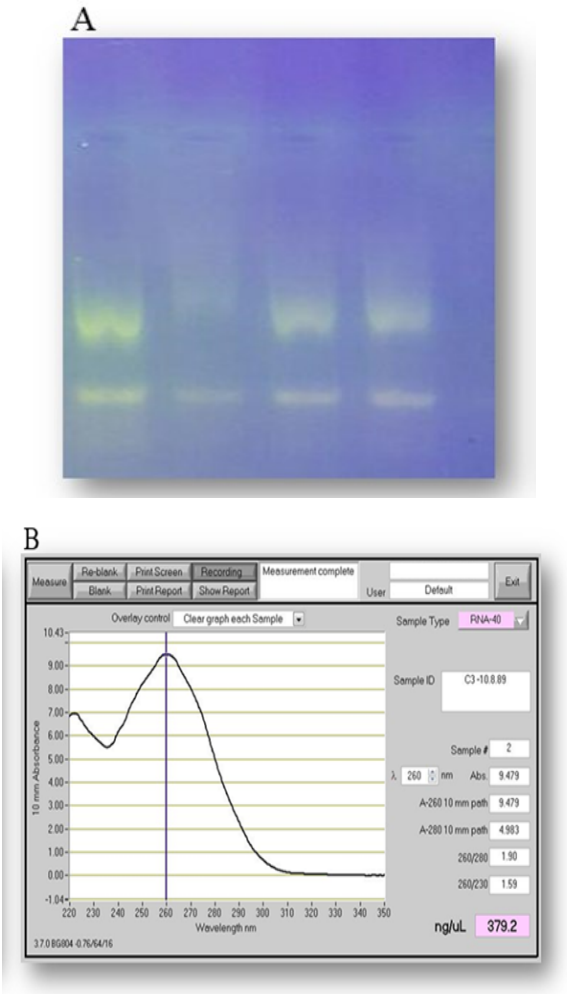

Figure1. RNA quantity and quality, A: Gel electrophoresis,B: Nanodrop

3.2 The expression levels of miRNA-601 and the U6 gene during the pathogenic stage of a disease

The study revealed a significant reduction in the serum expression level of miRNA-601 in patients diagnosed with fourth stage lung cancer, in comparison to the healthy control group ($p<0.001$) (Figure2). The results suggest that miRNA-601 may serve as a potential biomarker for lung cancer detection and monitoring.

3.3 Distribution of the population in terms of gender and smoke

In this study, 29 smokers and 11 non-smokers were randomly selected. Cigarette smoking is one of the factors that causes lung cancer. There was a significant correlation between miRNA-601 expression level and smoking status in lung cancer patients ($p=0.02$) (Figure3). The present investigation offers novel perceptions concerning the involvement of miRNA-601 in lung carcinoma, with emphasis on the significant role of smoking cessation as a prophylactic strategy for this fatal ailment (Figure3A). Also, the expression level of miRNA-601 was not significantly associated with gender (Figure3B).

4. Discussion

LC is a malignancy with dire consequences, often identified at an advanced stage, resulting in a grim prognosis and narrow therapeutic alternatives. The identification of dependable biomarkers for the purpose of early detection and monitoring of lung cancer holds significant importance. Recent years have seen significant developments regarding the potential utility of miRNAs as biomarkers for a range of malignancies, including lung cancer. Yu Yao et al investigated the role of microRNAs in gastric cancer(GC) in 2009, 847 microRNAs in GC of Chinese patients were analyzed by Real-Time PCR, the results of the analysis showed that 22 microRNAs differed significantly between normal and gastric cancer tissue [12].

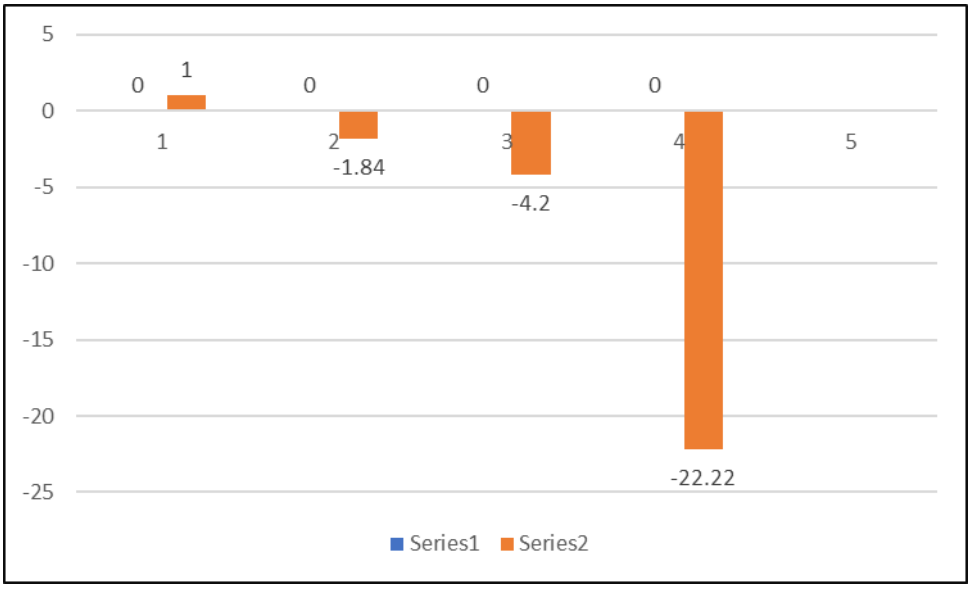

Figure2. The expression level of miRNA-601

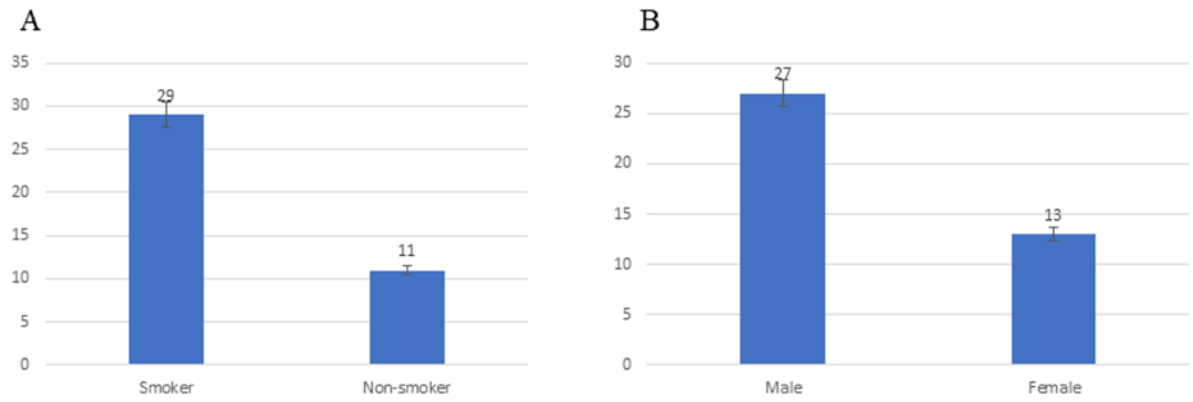

Figure3. The distribution of the population in terms of smoking and gender

In this study, we investigated the expression level of miRNA-601 in the serum of Iranian lung cancer patients and its relation to smoking. Our findings revealed that miRNA-601 expression was significantly decreased in lung cancer patients compared to healthy controls. This suggests that miRNA-601 may serve as a potential biomarker for metastatic lung cancer detection and monitoring.

The function of miR-601 as either an oncogene gene or a tumor suppressor gene can fluctuate depending on the particular form of cancer. An example of this can be seen in the research conducted by Cao et al. The research indicated that the expression of miR-601 was considerably decreased in samples of pancreatic cancer. Conversely, an increase in the expression of miR-601 led to a decrease in the proliferation and migration of cancer cells in the pancreas[13]. Hiroaki Ohara and colleagues in 2009 investigated the effects of microrna601 on gene expression in human A549 cancer cells, which showed that microRNAs play a role in cell signaling pathways and suppress NF-kappa factor, which plays a role in cell survival [11]. Qi feng wang et al in 2012 examined the expression of microRNA60 in CRC cancer and concluded that the expression of this microRNA was reduced [14]. Oncol Rep et al in 2013 compared the expression levels of microRNA601 in tumor and non-tumor tissues in esophageal squamous cell carcinoma. And concluded that microRNA601 expression was reduced in tumor tissues compared to non-tumor cells [11].

Cao W et al studied microRNA601 expression in pancreatic cancer and found that microRNA601 expression was decreased in PC tissue samples than in healthy individuals, especially in non-metastatic PC Metastasis [15].

Furthermore, our study revealed a correlation between miRNA-601 expression and smoking status in lung cancer patients. Smokers with lung cancer had significantly higher levels of miRNA-601 compared to non-smokers with lung cancer. This finding emphasizes the importance of smoking cessation as a preventive measure for lung cancer. Interestingly, the combination of miRNA-601 and smoking status improved the diagnostic performance for lung cancer. However, miRNA-601 expression was not significantly associated with gender.

Although our research offers significant findings regarding the possible connection between miRNA -601 and lung cancer as a biomarker, additional large-scale studies are necessary to authenticate these results. Moreover, the mechanisms that connect miRNA-601 expression with lung cancer and smoking must be investigated. In the future, it would be worthwhile to explore the potential application of miRNA-601 as a predictive indicator for lung cancer.

In conclusion

In conclusion, our study highlights the importance of identifying reliable biomarkers for early detection and monitoring of lung cancer. MiRNA-601 may serve as a potential biomarker for lung cancer detection and monitoring, and smoking cessation should be encouraged as a preventive measure for lung cancer.

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